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Microcontainers as an oral delivery system for spray dried cubosomes containing ovalbumin

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Abstract

The purpose of this study was to prepare cubosomes encapsulating the model antigen ovalbumin (OVA) via spray drying, and to characterise such cubosomes with a view for their potential application in oral vaccine delivery. Furthermore the cubosome formulation was loaded into polymeric microcontainers intended as an oral drug delivery system. The cubosomes consisted of commercial glyceryl monooleate, Dimodan®, containing OVA and were surrounded with a dextran shell prepared by spray drying. Cryo-TEM was used to confirm that cubosomes were formed after hydration of the spray dried precursor powder. The precursor powder had a mean particle size of 1.3±0.1 µm, whereas the mean diameter of the dispersed cubosomes was 282±7 nm (PDI: 0.18) measured by dynamic light scattering. 8.5±0.3 % (w/w) of OVA was present in the cubosome powder and OVA was found released slowly over the first 70 h, followed by a more rapid release. Total release of 47.9±2.8 % of loaded OVA occurred over 96 h in a buffer at pH 6.8. When the powder was filled into microcontainers, and the opening covered with the pH sensitive polymer Eudragit S100, the pH sensitive 'lid' was intact at gastric pH, but release of OVA from the cubosomes and microcontainers occurred at pH 6.8, releasing 44.1±5.6 % of the OVA in 96 h. Small-angle X-ray scattering (SAXS) revealed that the 'dry' particles possessed an internal ordered lipid structure (lamellar and inverse micellar phase) by virtue of a small amount of residual water, and after hydration in buffer at pH 6.8, the particles formed the hexagonal inverse cubic phases, thereby indicating that cubosomes were formed when released from microcontainers.

Introduction

Vaccination is often regarded as the most significant contribution to public health and disease prevention and moreover, it is a very cost-effective medical intervention [1,2]. Vaccination has reduced the morbidity and mortality resulting from diseases such as tuberculosis and smallpox and has thereby saved millions of lives. In spite of this, many infectious diseases remain endemic in large parts of the world, and therefore vaccination is an area in continuous development [1,2].

Most vaccines are administered by injection and there are only a few oral vaccines on the market such as rotavirus vaccine (as solution or suspension) and a capsule with vaccine formulation against typhoid fever [3]. Although, the oral route can be beneficial for vaccine administration [4,5]. Some of the advantages of oral vaccines are the ease of administration and an increased safety compared to injections. In addition, there is also a great potential for mass vaccination without the requirements of trained personnel [4,6]. Furthermore, oral vaccines have the ability to induce both mucosal and systemic immune responses [6,7], as shown in the 1990s with several HIV vaccines [8], and they are therefore considered ideal for combating infectious diseases. Although, oral vaccines have several attractive features, there are some major challenges.

The target of vaccine formulations in the gastro-intestinal (GI) tract is the M-cells in the intestine [9]. The antigen might be damaged, when passing through the harsh environment of the GI tract, which in turn will lead to the need for large doses. In addition, there is a poor transport of the antigen across the intestinal epithelium [4].

Traditional vaccines are mainly composed of heat-inactivated bacteria or viruses resulting in high immunogenicity. The risk with these types of vaccines is that they, in the body, can change to the active state and thereby infect the patients with the bacteria or virus and thus, leading to unwanted side effects [1,10]. Consequently, new generation vaccines are developed with subunit antigens. These subunit antigens are highly purified components of pathogens and thereby chemically well-defined. Hence, there is a much higher safety than for traditional antigens, but as the subunit antigens lack most of the features of the original pathogen they tend to be poorly immunogenic [1,10]. Therefore, to succeed with oral vaccine delivery, delivery systems need to be developed, in which the antigen can be encapsulated into particles [11,12]. These particles will assure presentation of the antigen to the antigen-presenting cells, but can also stabilise and release the antigen over an extended period of time [10]. Some particles will provide an adjuvant effect in themselves, but potent adjuvants can in addition also be added to the particulates for inducing an effective immunity [13].

There are many possibilities for vaccine delivery systems, and some of the most common ones are: polymeric micro- and nanoparticles, immunostimulatory complexes and liposomes [14,15]. Cubosomes have also shown to be an efficient delivery system for vaccines [11]. Cubosomes contain a highly twisted, continuous lipid bilayer with two congruent, non-intersecting water channels, giving the particles both hydrophobic and hydrophilic domains [11]. This offers great flexibility with respect to the types of compounds, that can be incorporated into the particles [16,17]. Rizwan et al. found that significantly higher amounts of antigen can be encapsulated in cubosomes compared to liposomes due to the larger surface area of cubosomes, and moreover in cubosomes, the antigen was also retained more efficiently compared to liposomes [11].

Traditionally, cubosomes are produced by mixing monoolein or phytantriol and water and thereby creating a high-energy dispersion followed by colloidal stabilisation using polymeric stabilisers [11,18,19]. However, it can be desirable to have the vaccine particles in a powder form (here termed “precursors”), and precursors of cubosomes have earlier been produced by either freeze drying [20] or spray drying [21–23]. In the spray drying process, dry powder precursors have the active ingredient incorporated, and upon hydration colloidally stable cubosomes are spontaneously formed. The powder form of the vaccine formulation can be advantageous in terms of stability of the antigen. Also, there is no need for a cold-chain storage which is needed for traditional vaccines [4].

After oral administration of the vaccine formulations, the antigen needs to be protected in the stomach and during transportation to the small intestine. In the small intestine, the vaccine particles should be delivered to the microfold (M) cells of the peyer’s patches as they will present the antigen to the underlying immune cells and thereby obtain an immune response [24]. The particles, carrying the antigen (and adjuvant), can give some protection of the antigen through the GI environment, but often the particles will also degrade on the way to the intestine, and therefore more advanced drug delivery systems can be necessary. An example of these advanced drug delivery systems is microcontainers. Microcontainers are polymeric, cylindrical devices in the micrometre size range (Fig. 1) [25–27]. They have the potential for targeted and/or sustained delivery in the GI tract [28]. Some of the advantages of the microcontainers are that size and shape can be controlled very precisely. Furthermore, the devices allow for unidirectional release, as only one side of the microcontainer is open, compared to more conventional microparticles where release can occur from the whole surface area. This has shown to increase the drug concentration at the microdevice-cell interface and thereby, allowing for increased permeation of the drug *in vitro* leading to enlarged oral bioavailability of the drug [27,29,30]. In addition, the antigen can be protected inside the cavity of the microcontainer from the harsh environment of the stomach until release is desirable [31,32]. The microcontainers have previously shown to interact with the intestinal mucus resulting in prolonged drug absorption [27]. It is reported in the literature that one way to improve oral vaccine delivery is to extend the intestinal residence time [13], hence, the microcontainers can be a promising platform for this purpose. In this paper, SU-8 (an epoxy photoresist) was used as a model polymer for fabrication of the microcontainers [25,26,31], but microcontainers have also been fabricated using biopolymers such as poly-L-lactic acid (PLLA) [33,34].

The aim of this study was, as a proof-of-concept, to prepare and characterise cubosomes loaded with ovalbumin (OVA) in a spray dried powder form for future application in oral vaccine delivery. The precursor powder was filled into microcontainers for protection and release control, and the *in vitro* release was studied together with small-angle X-ray scattering (SAXS) to confirm whether cubosomes were released from the microcontainers as internally structured particles.

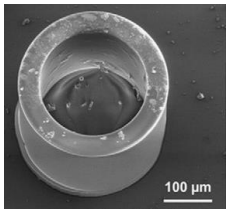

Fig. 1: SEM image of an SU-8 microcontainer with an inner diameter of 223 µm [26].

Materials and methods

Materials

OVA was purchased from TCI Europe (Zwijndrecht, Belgium). Dimodan® D90 was kindly donated by Danisco (Grindsted, Denmark). Dextran (from Leuconostoc Mesenteroides) and potassium dihydrogen phosphate were acquired from Sigma-Aldrich (St. Louis, MO, USA). Pierce BCA Protein Assay kit was purchased from Thermo Fisher Scientific (Rockford, IL, USA). Deionised water was obtained from an SG Ultra Clear water system (SG Water USA, LLC, Nashua, NH, USA) and was freshly produced in all cases. All other chemicals used were of analytical grade.

Spray drying of precursors for cubosomes containing OVA

Cubosomes were prepared using a commercial source of glyceryl monooleate (GMO), Dimodan® MO 90/D. The cubosomes were loaded with OVA as a model antigen, and the particles were surrounded by a dextran shell. The particles were prepared by first dissolving GMO in ethanol (1.78 w/v %), and then mixing with a solution of OVA in MilliQ water (0.075 final w/w % of OVA). After 1 h of mixing, dextran dissolved in MilliQ water (1.77 w/v %) was added to the GMO/OVA solution (0.72 w/w % of GMO + OVA), and the final solution was spray dried using a B 290 Büchi mini spray dryer (Büchi Labortechnik AG, Flawil, Switzerland). Free OVA was not removed prior to the spray drying process.

For the spray drying of the precursors, a 0.7-mm nozzle was used and air was utilised as the drying medium. Spray drying was performed at an inlet temperature of 200 °C resulting in an outlet temperature of approximately 85 °C. The drying flow rate was set to 32 m^3/h and an aspirator capacity of 80 % with a feed rate of 4 mL/min was used. Particles without OVA were also produced as blank particles and used as reference.

Cryo-TEM of cubosomes

The precursors for the cubosomes with OVA were dispersed in MilliQ water at a concentration of 1 mg/mL. The samples for the Cryo-TEM studies were prepared in a controlled environment vitrification system (CEVS). A small amount of the sample (5 µL) was put on a carbon film supported by a copper grid and blotted with filter paper to obtain a thin liquid film on the grid. The grid was quenched in liquid ethane at −180 °C and transferred to liquid nitrogen (−196 °C). The samples were then examined using a Tecnai G2 F30 Transmission Electron Microscope (FEI, Eindhoven, The Netherlands) operating at a voltage of 300 kV and a working temperature of −180 °C. Images were recorded using Gatan UltraScan 1000 (2k × 2k) CCD camera (Gatan, California, USA).

Size of particles

The size of the dry particles with the dextran shell was measured using aerosizer particle size analyser (Model 3321, TSI Incorporated, MN, USA) by setting the pump to 1.37 bar and with a capillary flow of 8 L/min. A small amount of powder was distributed on the plate and the particle size was measured in six replicates.

For the particles dispersed in water, the particle size distribution (Z-average), polydispersity (PDI) and zeta potential were determined using dynamic light scattering (Malvern Zetasizer, NanoZs ZEN 3600, Malvern, UK). Measurements were performed at 37°C, and the results presented are the mean of three successive

measurements of 100 s of at least three independent samples. Samples were diluted with water to adjust the signal level.

OVA present in the cubosomes

Precursor powder (10 mg) was added to a solution of 20 mM phosphate buffer, pH 6.8 containing 5 % Triton X-100. After vortex mixing, the cubosomes were dissolved and a sample of 200 µL was taken out. A BCA Protein Assay kit was used to determine how much OVA was present in the cubosome powder by following the procedures for the standards and samples recommended by the manufacturer. The same process was performed with the blank cubosomes to check for any cross activity of the formulation. The absorbance was measured at 562 nm on a plate reader, and the obtained absorbance values were analysed against the standard curves prepared on the same day as the samples. OVA entrapment was then determined by calculating the difference between the total OVA added before the spray drying process and the free fraction of OVA in the solution. The experiments were performed in triplicates.

Fabrication of SU-8 microcontainers

Production of the microcontainers involved two steps of photolithography with the negative epoxy-based photoresist, SU-8 [26,32]. The microcontainers were structured on a fluorocarbon coating deposited on top of the supporting silicon wafer by plasma polymerisation. This enabled dry removal of the fabricated SU-8 devices from the support substrate in order to obtain individual microcontainers if needed [27,35]. The fabricated microcontainers had an inner diameter of 223±3 µm and a height of 270±3 µm (mean±SD, n=6). Silicon wafers supporting the microcontainers were finally cut into squares of 12.8 x 12.8 mm^2 using an Automatic Dicing Saw from DISCO (Kirchheim b. München, Germany). Each chip contained arrays of 25 x 25 containers with a pitch of 450 µm.

Filling of microcontainers with powder precursors

Powder precursors were manually distributed on the microcontainer chip. The excess drug in between the microcontainers was then removed with pressurised air, resulting in powder-filled microcontainers [27]. The chip with microcontainers was weighed before and after filling to determine the amount of drug filled into the microcontainers.

Spray coating of the filled microcontainers with Eudragit S100

A spray coating system (ExactaCoat, Sono Tek, USA) equipped with an ultrasonic nozzle actuated at 120 kHz [36] was used to deposit Eudragit S100 (dissolved to a 2 % (w/w) solution in isopropyl alcohol) on the cavity of the drug-filled microcontainers in a set-up similar to previously described [33]. The generator power was set to 1.5 W, and the polymer solution was pumped through the nozzle at a flow rate of 100 µL/min. Nitrogen gas at a pressure of 10 mbar was used to direct the beam of droplets onto the microcontainers, and the distance between nozzle and substrate was 40 mm with the beam diameter on the substrate being approximately 4 mm. The lateral movements of the nozzle were controlled by an x-y stage and the nozzle path was defined in the equipment software. The nozzle was moved line-by-line at a speed of 25 mm/s, and the coating was repeated 60 times to obtain a coating thickness in the µm range.

Release of OVA from the cubosomes

In vitro release of OVA from the cubosomes unconfined (bulk powder) and confined in microcontainers coated with Eudragit S100 was investigated on a µDISS profiler (*p*ION INC, Woburn, MA). In both release studies, each channel was calibrated with its own OVA standard curve prior to the experiments. For the calibration curves, aliquots of OVA in water stock solution were repeatedly added to 10 mL of either a HCl solution or a phosphate buffer in order to achieve a range of defined standard concentrations, and the UV spectrum of each standard was recorded. The release experiments were performed at 37±0.5°C using a stirring rate of 200±5 rpm using 20 mm path length *in situ* UV probes on a µDISS profiler. The absorbance data was evaluated using 280 nm on the standard curve and utilising the 2nd derivative function in the Au Pro software affiliated with the µDISS profiler.

The release of OVA from the precursor powder was studied in 20 mM phosphate buffer, pH 6.8 for 96 h. The *in situ* UV probes were situated in each sample vial containing 10 mg of powder and 10 mL of phosphate buffer was added. The probes scanned and detected the absorbance of released OVA.
The release studies from the microcontainers were performed in a set-up similar to one previously described [25,27,33]. The chips with microcontainers were attached to cylindrical magnetic stirring bars (using carbon pads) and placed in the bottom of sample vials. The chips were covered with 10 mL of 0.1 M HCl pH 1.6 for 2 h and subsequently, the medium was changed to 10 mL of 20 mM phosphate buffer, pH 6.8 for 96 h, and the *in situ* UV probes detected the absorbance.
Both sets of experiments were performed in 3 replicates.

Scanning electron microscopy of the microcontainers

SEM was utilised to examine the microcontainers after filling, after spray coating of the lid of Eudragit S-100, and after release in phosphate buffer at pH 6.8. The examinations were carried out using a Phenom Pro scanning electron microscope (Phenom World, Eindhoven, the Netherlands). Prior to the investigations, the microcontainer chip was mounted onto metal stubs, and imaging was performed at an operation voltage of 10kV with a 600x magnification.

SAXS determination of the structure of cubosomes loaded into microcontainers

The SAXS/WAXS beamline at the Australian Synchrotron, Clayton, Australia [37] was used to determine the internal structure of the spray dried particles, when the cubosomes were confined in microcontainers and released from the devices. The X-ray beam had an energy of 11 keV, and the 2D SAXS patterns were collected using a Pilatus 1M camera (active area 169 x 179 mm^2 with a pixel size of 172 x 172 µm), which was located 900 mm from the sample position. The total q range for the instrument configuration outlined above was $0.02 < q < 1.06$ Å^{-1}, and 2D SAXS patterns were collected for 1 sec. The in-house designed computer software 'ScatterBrain' was used to acquire and reduce these 2D patterns to 1D intensity versus q profiles. The powder-filled microcontainers were separated from the base using a scalpel, and filled into a 1.5 mL capillary and SAXS patterns were acquired in dry state followed by addition of 50 µL of MilliQ water, where after patterns were acquired for a time period of 80 min. The set-up with empty microcontainers as an example can be seen in Fig. 2A, with an image of the microcontainers in a capillary in the X-ray beam shown in Fig. 2B.

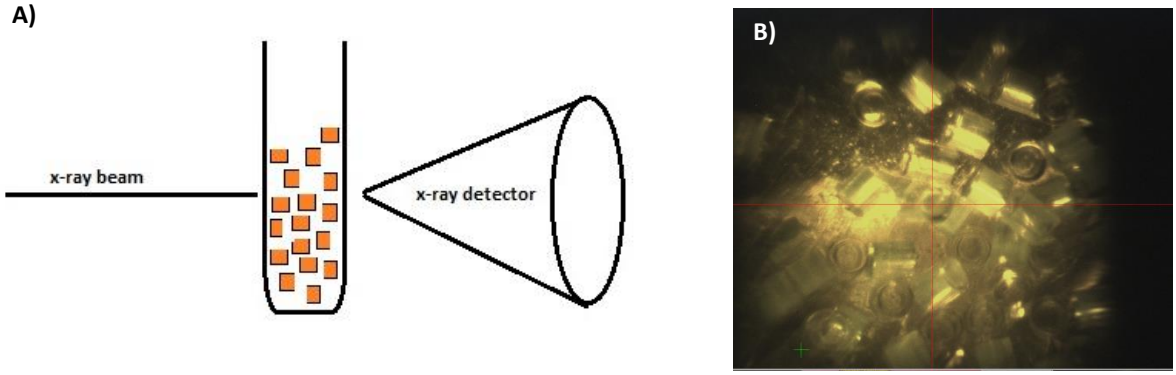

Fig. 2: A) Schematic of the SU-8 microcontainers filled into a capillary to be used in the SAXS/WAXS synchrotron. B) Micrograph showing the set-up with the microcontainers in the x-ray beam.

Statistics

The data are expressed as mean ± standard deviation (SD). Where appropriate, statistical analysis was carried out using Student t-tests using GraphPad Prism version 7.00 (GraphPad Software Inc., CA, USA). P-values below 5 % ($p < 0.05$) were considered statistically significant.

Results and discussion

For the production of the powder precursors of cubosomes, spray drying was chosen as this is a simple technique converting a solution to powder in a one-step process [38]. GMO has for many years been one of the lipids of choice for producing cubosomes, as it is non-toxic, biocompatible and biodegradable [39], and therefore it was decided to produce GMO particles in this study. The spray drying technique is convenient for producing the powder precursors, but GMO can be challenging to spray dry as it immediately forms the cubic phase upon hydration. Spicer et al. studied the effect of applying ethanol as a hydrotrope and this resulted in the formation of a low-viscous emulsion that was easily spray dried [21]. For this reason, in this study, GMO was first dissolved in ethanol and then added to the aqueous dextran solution. It has been reported that GMO itself produces sticky agglomerates after spray drying, and to obtain a more flowable powder an aqueous starch or a dextran solution can be added prior to spray drying resulting in the GMO being encapsulated in a dry starch or dextran shell [21–23]. In this work, it was chosen to add dextran as the anti-cohesion agent, and the produced powder was flowable and easy to hydrate. After production of the GMO powder precursors, the powder was hydrated and cryo-TEM was performed to identify whether cubosomes were obtained. It can be observed in Fig. 3 that cubic structures were found after hydration of the powder.

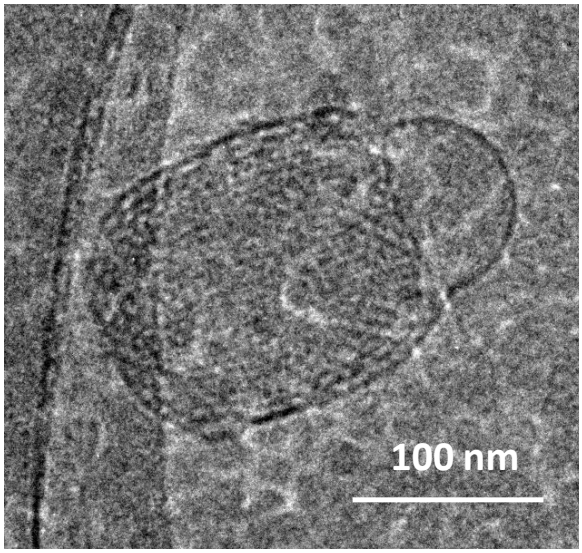

Fig. 3: Cryo-TEM image of a representative hydrated particle with a distinct cubic liquid crystalline structure. The resolution in cryo-TEM images is limited by the presence of dissolved dextran.

In vitro characterisation of the particulates

The size, shape and surface charge of a particulate vaccine carrier will influence its performance as a vaccine [40]. The dry powder with OVA and the dextran shell had a size of 1.3±0.1 µm, whereas the dry blank particles without OVA had a size of 1.6±0.1 µm. After hydration, self-assembled, close to neutrally charged nanoparticles were formed, with mean size of 146.1±1.3 nm and 281.7±7.4 nm for the blank and OVA-loaded particles, respectively (Table 1). There was a significant size difference between the blank and OVA-loaded particles (p-value <0.0001), and the PDI for both formulations was low, indicating homogeneous formulations. The particles were much smaller than those reported by Spicer et al., where the dry particles had a diameter of 24 µm, and in the hydrated form the cubosomes were in average 0.6 µm with a size distribution from 0.1 to 5 µm [22]. In general, it is reported that the particle size should be between 20 nm to 10 µm to be well recognised by the immune system [11], but more specifically for oral vaccine formulations, a size between 200-500 nm can be advantageous for uptake into the antigen-presenting cells after oral administration [40,41]. In relation to this, it can be observed that the size of the cubosomes with OVA is in this size range, and the cubosomes should therefore be able to be taken up by the antigen-presenting cells.

Table 1: Size measurements of the hydrated cubosomes with and without OVA dispersed in MilliQ water. The measurements were performed using dynamic light scattering in triplicates, and data are represented as mean±SD

	Z-average (nm)	**PDI**	**Zeta potential (mV)**
Blank particles	146.1 ± 1.3	0.15±0.02	-0.43±0.077
Particles with OVA	281.7 ± 7.4	0.18±0.11	-0.18±0.042

Presence and release of OVA in and from the particles

Before the release measurements, it was initially determined that 8.5±0.3 % (w/w) OVA was present in the cubosome powder. It is also well-known that cubosomes often provide a sustained release of a drug [39], and this is also observed in this study, where release studies in buffer at pH 6.8 showed that during the first

70 h, OVA was slowly released, followed by a more rapid release from 70-80 h. A total release of 47.9±2.8 % was observed in relation to the total loading of OVA in the cubosomes over a 96 h period (Fig. 4). It can be seen that there is a significant burst release of OVA from the cubosomes (insert in Fig. 4) of 18.2±1.6 % in the first 10 min. This is probably caused by the release of OVA from the powder just when the powder precursors are dispersed in the aqueous solution, and thereafter the OVA entrapped in the channels of the cubosomes is released. In the literature, it is reported that OVA was released during 168 h from cubosomes resulting in a complete release [18]. A study preparing precursors of cubosomes by spray drying, but encapsulating the highly lipophilic drug, efavirenz, also is reporting on a burst release of the drug of up to 16 %, with a total release in 12 h of up to 56 %, again indicating that when dispersing powder precursors in aqueous solution a burst release is occurring [23]. A sustained release of OVA is also observed in this study and this could be beneficial when developing vaccine formulations [42].

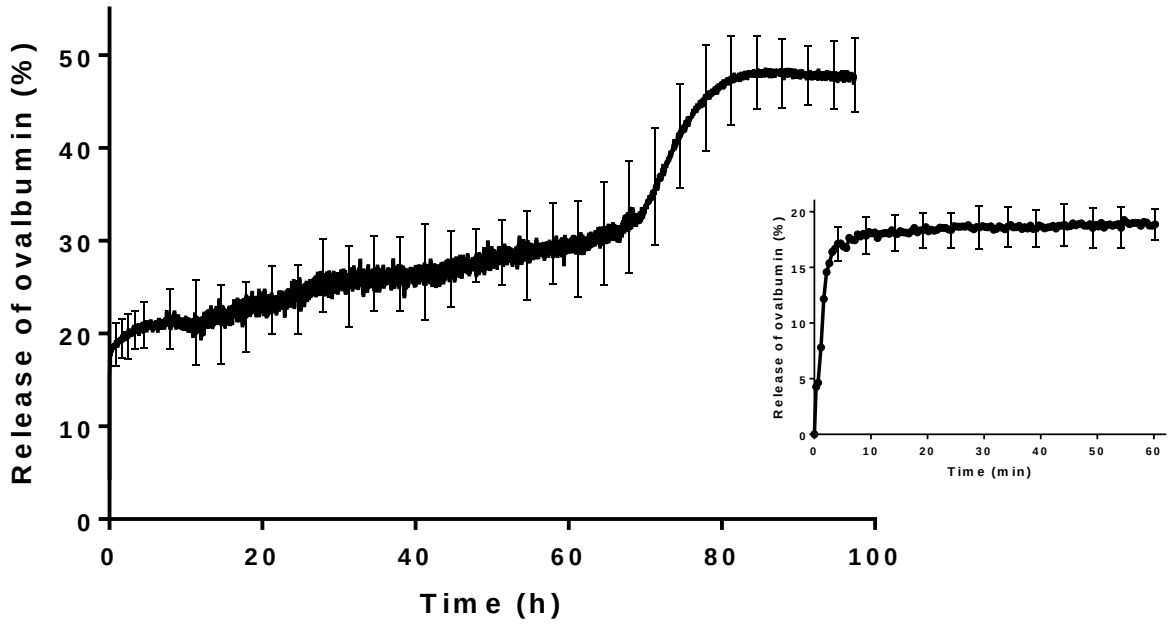

Fig. 4: Release of OVA from the cubosomes in 20 mM phosphate buffer pH 6.8, expressed as % of the total content of OVA. The insert is showing the release over the first 60 min. The release study was performed in triplicates, and the data represent mean±SD.

Internal structure of particles formed upon hydration from microcontainers

SAXS/WAXS can be used to detect phase transformations in self-assembled lipid systems, and this was utilised to identify whether particles released from the microcontainers contained internal nanostructures consistent with cubosomes. Fig. 5 shows the plot of intensity versus the scattering vector q obtained from the release of GMO particles in dry form and when the microcontainers containing the particles were dispersed in water for a period of 80 min. For the dry particles, it can be observed that there are three equally spaced peaks in the diffractogram (Fig. 5), indicating that the dry particles are in a lamellar phase with the lattice parameter of 49.5 Å (Table 2). There is also an inverse micellar phase present, indicated by the broad peak at q ~ 0,2 $Å^{-1}$ in the diffractogram) with a D-spacing of 31.1 Å. This can be explained by the

presence of residual moisture in the spray dried powder. According to the phase diagram of GMO in water, the inverse micellar phase and lamellar phase coexist at approximately 5 % water [43,44], consistent with Spicer et al. reporting approximately 5 % (w/w) of moisture content in their spray dried cubosome powders [22].

After hydration (here exemplified by the diffractogram at 50 min), the liquid crystalline nanostructured particles showed a mix of phases, with peak indexing indicating coexisting inverse hexagonal (H_2) phase (peaks at √1 : √3 : √4), . Pn3m cubic phase (peaks at √2 : √3 : √4), and Ia3d cubic phase (peaks at √6 : √8). These three phases appear in the GMO + water phase diagram [44], and the calculated lattice parameters are listed in Table 2. The presence of H_2 and Pn3m cubic phases for commercial GMO samples in water might be expected at full hydration, however the Ia3d cubic phase is only expected at less than full hydration o the lipid. Therefore it is proposed that the particles were not completely hydrated after 50 min, which is also supported by the fact that after 80 min the Ia3d phase appears even less prominent.

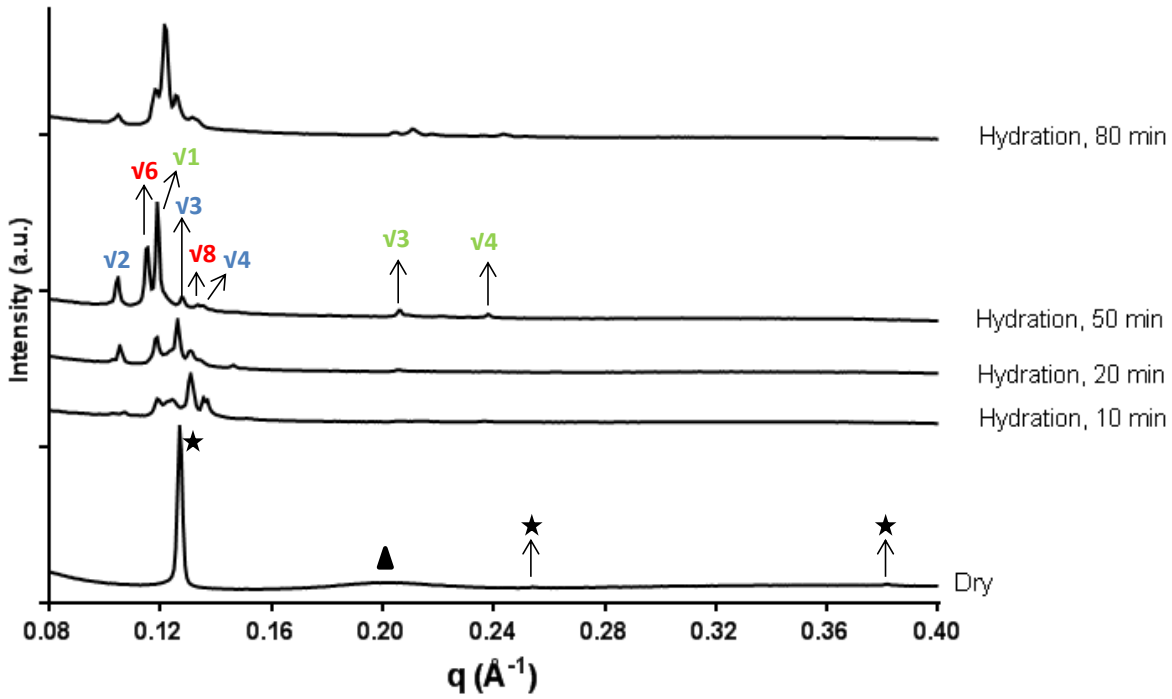

Fig. 5: 2D SAXS patterns were collected from cubosomes confined in microcontainers and followed while the cubosomes were released from the microcontainers in MilliQ water. The cubosome filled microcontainers were enclosed in a glass capillary during hydration for up to 80 min. After 50 min of hydration the particles show a mix of phases with inverse hexagonal (H_2) phase (peaks at √1 : √3 : √4), Pn3m cubic phase (peaks at √2 : √3 : √4), and Ia3d cubic phase (peaks at √6 : √8).

Table 2: Phase structure and lattice parameters obtained from SAXS measurements of dry particles and particles released from microcontainers after hydration, here with an example after 50 min of hydration.

	Lattice parameters (Å)	
	Dry particles	**Hydration for 50 min**
Lamellar phase, L_α	49.5	
Inverse micellar phase, L_2	31.1	
Inverse hexagonal phase, H_2		61.0
Inverse bicontinuous cubic phase, Pn3m		84.6
Inverse bicontinuous cubic phase, Ia3d		132.7

Loading of precursors into the microcontainers and coating of the pH sensitive lid

After successfully loading the cubosomes into the microcontainers (Fig. 6A), the cavity of the microcontainers was coated with Eudragit S100 (Fig. 6B) as this polymer will dissolve at a pH value of approximately 7 corresponding to the pH found in the small intestine around the M cells [9].

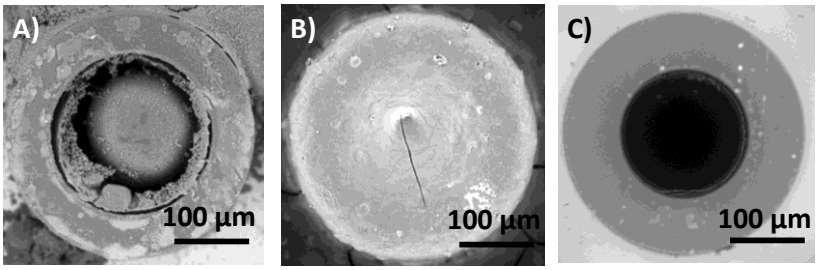

Fig. 6: SEM images of A) a cubosome-filled microcontainer, B) a filled microcontainers with a lid of Eudragit S100, and C) an empty microcontainer after release study in phosphate buffer pH 6.8.

Release of OVA loaded cubosomes from coated microcontainers

The coating on the cavity of the microcontainers can prevent the release until the intestine [33], and a large dose (approximately 2 µg of powder) of the vaccine formulation can be loaded into the cavity of the microcontainers [27]. In the study, the cavity of the microcontainers was coated with the pH sensitive polymer Eudragit S100. The release of OVA from the cubosomes and microcontainers was first measured for 2 h in a pH value corresponding to the pH of the stomach (pH 1.6), and here, as expected, no release was observed due to the intact layer of the Eudragit lid (Fig. 7). After 2 h, the pH of the medium was changed to reflect that of the small intestine (pH 6.8). Fig. 7 shows that the release of OVA is occurring, and this indicate that the cubosomes are also released from the microcontainers as these are empty after the release studies (Fig. 6C). The release is appearing in a more controlled fashion than observed from the unconfined powder cubosomes (Fig. 4). The OVA release in pH 6.8 is 44.1±5.6 % in relation to the amount of OVA in the particles. This is comparable to the release from the bulk powder being 47.9 % after 96 h (p-value: 0.4311).

In the literature, a rice-based oral vaccine has shown to be efficient as a delivery system as it can protect the antigen from enzymes in the stomach [7]. The microcontainers have the same feature and therefore, there is a promise for the microcontainers to work as an oral vaccine system as well. When delivering vaccines by the oral route, the delivery system should be able to present the vaccine formulation to the M cells followed by transport to the immune cells to create a response. It has been shown to be effective to

keep the vaccine formulation inside a particle for a significant period of time [10,12], securing a slow release. Therefore the slow release that the microcontainers and the cubosomes provide can be a great advantage when delivering vaccines.

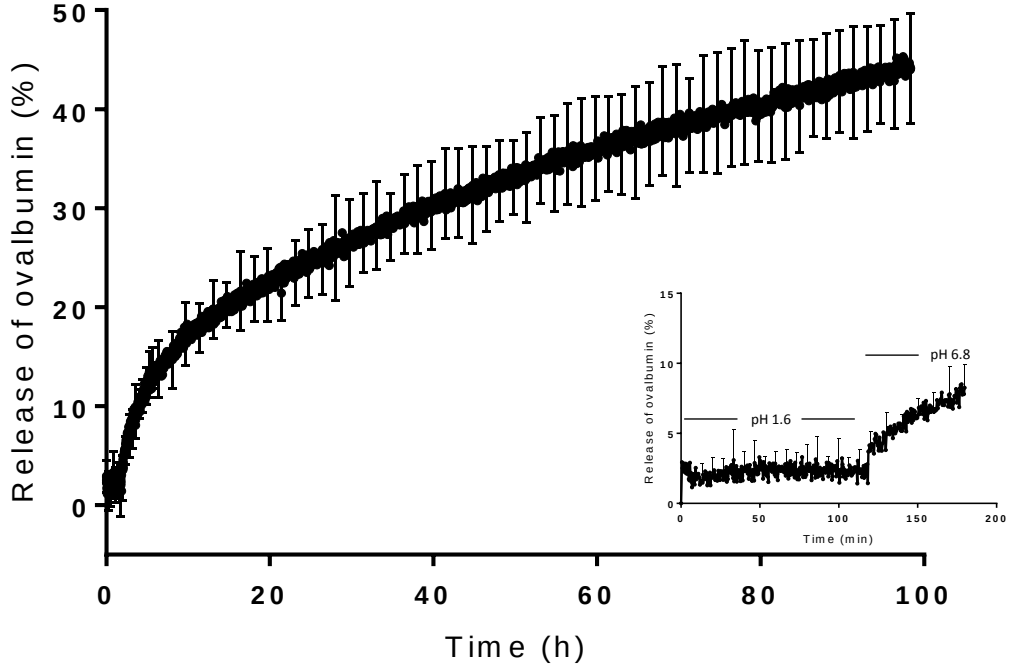

Fig. 7: Release of OVA from the cubosomes, when the vaccine formulation was confined in microcontainers. The release of OVA is expressed as a % of the loaded OVA into the cubosomes. For the first 2 h the release was measured in pH 1.6 followed by pH of 6.8 for up to 98 h. The data is presented in triplicates as a mean±SD.

Conclusion

Powder precursors of cubosomes loaded with OVA have been produced by spray drying, and it was concluded that the precursors contained cubic structure in bulk as well as when released from microcontainers. The microcontainers coated with an Eudragit S100 lid can serve as an oral vaccine delivery system protecting the cubosomes through the GI tract until release occurs in the small intestine. For these produced cubosomes to be completely developed as an oral vaccine system, an adjuvant needs to be added to the particles to obtain the optimal effect of this system and further investigations are therefore also needed for fully develop this oral vaccine delivery system.

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